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Brief cognitive training interventions in young adulthood promote long-term resilience to drug-seeking behavior

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Abstract

Environmental stress and deprivation increase vulnerability to substance use disorders in humans and promote drug-seeking behavior in animal models. In contrast, experiences of mastery and stability may shape neural circuitry in ways that build resilience to future challenges. Cognitive training offers a potential intervention for reducing vulnerability in the face of environmental stress or deprivation. Here, we test the hypothesis that brief cognitive training can promote long-term resilience to one measure of drug-seeking behavior, cocaine conditioned place preference (CPP), in mice. In young adulthood, mice underwent cognitive training, received rewards while exploring a training arena (i.e. yoked control), or remained in their home cages. Beginning 4 weeks after cessation of training, we conditioned mice in a CPP paradigm and then tested them weekly for CPP maintenance or daily for CPP extinction. We found that a brief 9-day cognitive training protocol reduced maintenance of cocaine CPP when compared to standard housed and yoked conditions. This beneficial effect persisted long after cessation of the training, as mice remained in their home cages for 4 weeks between training and cocaine exposure. When mice were tested for CPP on a daily extinction schedule, we found that all trained and yoked groups that left their home cages to receive rewards in a training arena showed significant extinction of CPP, while mice kept in standard housing for the same period did not extinguish CPP. These data suggest that in early adulthood, deprivation may confer vulnerability to drug-seeking behavior and that brief interventions may promote long-term resilience.

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Disclosures

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Keywords

cognitive training; conditioned place preference; cocaine; resilience; addiction

1. Introduction

Environmental conditions of deprivation and stress confer vulnerability to substance use disorders in humans and drug-seeking behavior in animal models (Buu et al., 2009; Enoch, 2011; Gordon, 2002; Lu et al., 2003; Meaney et al., 2002; Nader et al., 2012; Pilowsky and Wu, 2006; Sinha, 2008). In contrast, experiencing control over one's environment can buffer the effects of adversity, shaping neural circuitry in ways that promote resilience to future challenges and reduce later responses to drugs of abuse (Amat et al., 2010; Amat et al., 2008; Amat et al., 2006; Christianson et al., 2008; Kubala et al., 2012; Rozeske et al., 2012; Rozeske et al., 2011; Varela et al., 2012). Interventions that provide a sense of mastery, stability and control over one's environment may therefore promote resilience to substance use disorders.

Cognitive training that allows subjects to engage in active learning processes, forming and testing hypotheses about their environment based on feedback they receive (Gureckis and Markant, 2012; Lagnado and Sloman, 2004; Markant and Gureckis, 2014; Sobel and Kushnir, 2006), may serve as an effective intervention for providing a sense of control and stability in the face of environmental stress/deprivation. Relatively brief periods of cognitive training have been shown to shape neural circuitry in both humans and animals models (Haut et al., 2010; Johnson, 2014; Jolles et al., 2013; Mackey et al., 2013; Olesen et al., 2004; Subramaniam et al., 2012; Subramaniam et al., 2014; Takeuchi et al., 2010). We hypothesized that these changes in neural circuitry may influence animals' responses to future challenges. In particular, we hypothesized that a brief cognitive training intervention could promote resilience to drug-seeking behavior in mice raised in conditions of relative deprivation.

When rodents are removed from standard laboratory housing in which they have access to *ad libitum* food and water but have little opportunity for exploration and are placed in an enriched environment with greater cage size, playmates and toys, this enrichment has been shown to reduce vulnerability to drug-seeking behavior (Chauvet et al., 2012; Chauvet et al., 2009; Solinas et al., 2008; Solinas et al., 2009; Thiel et al., 2009). Upon returning to standard housing, however, animals with a history of enrichment show exacerbation of drug-seeking behavior (Nader et al., 2012) or no change in drug-seeking behavior (Chauvet et al., 2012) compared to animals with no history of enrichment. Permanent exposure to environmental enrichment is difficult to implement as a clinical intervention. We therefore tested whether a brief cognitive training intervention could promote long-term resilience to drug-seeking behavior even after animals returned to the relative deprivation of standard laboratory housing.

We employed a cognitive training paradigm in which mice learned arbitrary associations through trial and error, discriminating among multiple sensory stimuli in order to recover a buried food reward. Each trained mouse was paired with one yoked mouse, which explored

an adjacent arena and received a food reward each time the trained mouse earned a food reward. An additional group of mice remained in their home cages without cognitive training or food rewards, experiencing the relative deprivation of standard housing. Importantly, all groups of mice remained in their home cages for 4 weeks after cessation of cognitive training before cocaine exposure, allowing us to test the long-term effects of cognitive training on drug-seeking behavior after animals returned to standard housing. After this 4-week 'rest' period, mice underwent a cocaine conditioned place preference (CPP) protocol, which provided a measure of drug-seeking behavior. In our first experiment, we examined maintenance of drug-seeking behavior with weekly exposure to the CPP testing arena. In our second experiment, we examined extinction of drug-seeking behavior using daily exposure to the CPP testing arena (Mueller and Stewart, 2000).

All groups developed comparable preference for the cocaine paired chamber on the first CPP test day, but their behavior diverged with repeated exposure to the cocaine paired chamber. We found that cognitive training showed protective effects above and beyond the yoked control condition in weekly tests of maintenance of preference. In tests of extinction driven by daily exposure to the chamber, we found that all trained and yoked groups that left standard housing for an arena and reward showed significant extinction of cocaine CPP, while cage mates that were kept in standard housing for the same period did not extinguish CPP. These data suggest that in early adulthood, deprivation may confer vulnerability and that brief interventions may provide resilience to later substance abuse.

2. Materials and Methods

2.1. Experimental subjects

74 adult male C57BL/6 mice (Charles River Laboratories, bred in-house) were weaned at postnatal day (p)21 and housed with littermates in groups of 2 to 5. Cages contained plastic domes and nesting material and were kept on a reverse 12hr light/dark schedule. Mice were food restricted to 85–90% of their *ad libitum* body weights for 2 weeks during cognitive training but had *ad libitum* access to food and water at all other times. All animal procedures were approved by the Ernest Gallo Clinic and Research Center Institutional Animal Care and Use Committee and were consistent with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

All subjects received the same drug exposure. Cocaine HCl (Sigma-Aldrich) (10mg/kg) and saline (in equivalent volume) were administered via intraperitoneal (i.p.) injection on alternating days for 8 days.

2.3. Procedures for Experiment 1: Effects of cognitive training on weekly CPP maintenance

2.3.1 Training groups—Littermates housed together were divided into 3 groups: trained, yoked to trained (YT), and standard housed (SH). Trained animals underwent the 9-day cognitive training protocol described below and in Fig. 1, while YT littermates explored separate training arenas and received cereal rewards on a schedule yoked to that of trained animals. SH animals were food restricted to 85–90% of their *ad libitum* body weight during

the cognitive training period but did not receive any training or exposure to the training arena.

2.3.2. Cognitive training protocol—Mice were ages p55–p77 at the start of cognitive training. During training, mice learned to dig for cereal rewards (Honey Nut Cheerio pieces, General Mills, Minneapolis, MN) using odors, textures, and location as cues. The apparatus and task design exploited mice's natural foraging abilities to enable rapid learning of the task (Birrell and Brown, 2000; Johnson and Wilbrecht, 2011). The general training method and apparatus details are outlined in Johnson and Wilbrecht (2011) and are described more briefly here.

The apparatus included a start compartment, in which the mouse waited between trials, and digging compartments. Each digging compartment contained a digging pot filled with scented wood shavings (Hartz Mountain Corporation, Secaucus, NJ) and covered in a distinct texture. Only one digging pot contained an accessible cereal reward (approximately 10mg) in each trial, though all digging pots were pseudobaited with a whole Cheerio secured beneath a mesh screen on the bottom of the pot.

The odors used to scent the wood shavings (Table 1 and Table 2) were purchased from Alfa Aesar (Ward Hill, MA), San Francisco Massage Supply Co. (San Francisco, CA), and McCormick & Company (Sparks, MD). The scented liquids were diluted 1:10 in mineral oil or 50% ethanol and mixed with wood shavings at a concentration of 0.02ml/g. The textures used to cover the acrylic digging pots (Table 1) were purchased from local discount stores.

Each trial began with opening of the start gate, after which the mouse was allowed to explore freely but only dig in one pot per trial. Digging was defined as moving the wood shavings with both front paws. If a correct choice was made, the mouse was allowed to consume the cereal reward before being guided back to the start compartment. If an incorrect choice was made, the mouse was guided back to the start compartment. If the mouse did not dig within three minutes, the trial was terminated. The number of trials per session was held constant for all mice independent of performance. Trial number was set for each phase (see Fig. 1 caption) based on previous experiments and approximated the average number of trials that p60 male mice take to make 8/10 correct choices (Johnson and Wilbrecht, 2011). The 9-day training protocol proceeded as shown in Fig. 1 and included 3 rule reversals, 2 extradimensional shifts, and 1 intradimensional shift. Reversals and set-shifts were included in the training in order to engage executive functions such as cognitive flexibility and inhibitory control of behavior (Birrell and Brown, 2000; Johnson and Wilbrecht, 2011; Kim and Ragozzino, 2005; McAlonan and Brown, 2003). The specific odor/texture combinations used in each phase of training are listed in Table 1 and Table 2.

For the yoked control condition (i.e. YT mice), each YT mouse was paired with one trained mouse and was placed in a separate training arena during the trained mouse's sessions. Each time the trained mouse received a cereal reward, a comparable cereal reward was dropped into the arena of the YT mouse.

2.3.3. Measures of cognitive training performance—We calculated an overall performance score for each mouse using the fraction of trials resulting in a correct choice (correct trials/total trials) during sessions that involved rule changes (i.e. all sessions except compound discrimination and overtraining). The fraction of trials resulting in a correct choice was calculated for each session and then averaged across sessions. Performance scores (correct trials/total trials) were also calculated specifically for reversals (reversal 1, reversal 2, and reversal 3), extradimensional shifts (texture and spatial), and intradimensional shift (see Fig. 1).

To measure the mice's ability to inhibit responding to a previously rewarded cue, we measured perseverative errors, defined as errors before the first rewarded trial during reversal sessions (reversal 1, reversal 2, and reversal 3). To measure the mice's ability to adapt to a new contingency, we measured regressive errors, defined as errors after the first rewarded trial in any session involving a rule change (i.e. all sessions except compound discrimination and overtraining). Perseverative and regressive error indices were calculated as the fraction of trials resulting in each error type.

2.3.4. Conditioned Place Preference—Cocaine conditioning began 4 weeks after completion of cognitive training (Fig. 3A) and took place during the animals' dark cycle. The conditioned place preference (CPP) apparatus consisted of a plexiglass open field (Med Associates) divided into two chambers (27cm × 13cm × 20cm). The two chambers were distinguished by visual cues (horizontal vs. vertical bars on wall) and floor texture (pebbled vs. square pattern). Mouse movement was monitored by infrared beam breaks (Activity Monitor, Med Associates). Each animal was habituated to handling for 3 days prior to the start of the experiment. Habituation and conditioning took place over 9 weekdays, with the first CPP test day on the 10^{th} weekday.

Habituation: Mice were allowed to explore both chambers for 20 minutes, and their baseline preference for one of the two chambers was measured.

Conditioning: Mice received cocaine (10mg/kg) and saline injections on alternate days for 8 weekdays (4 injections of each drug). Immediately after the injections, mice were placed in one of the two chambers for 15 minutes with no access to the other side. Mice received saline in the initially preferred chamber (determined on habituation day) and cocaine in the non-preferred chamber.

CPP testing: Mice were next tested for chamber preference on post-conditioning days 1, 7, 14, 21, and 28, with day 1 being the first day after completion of cocaine/saline conditioning. We chose a weekly testing schedule based on previous literature showing that infrequent exposure to the drug-associated context can maintain CPP over long time periods in rodents (Mueller et al., 2002; Mueller and Stewart, 2000; Solinas et al., 2008). The weekly schedule allowed us to test long-term effects of cognitive training under conditions in which control animals would be expected to maintain cocaine CPP.

On each test day, mice received mock injections (i.e. handling with no needle puncture) and were allowed to freely explore either chamber for 20 minutes. CPP values were calculated as

the number of seconds spent in the cocaine-paired chamber minus the number of seconds spent in the saline-paired chamber. All CPP values were normalized to habituation preference by subtracting each mouse's CPP score on habituation day from its own CPP values on all other days. Mice that failed to achieve normalized CPP levels of 100 seconds on test day 1 were excluded from the study (1 trained mouse, 3 YT mice, and 1 SH mouse) due to questions about the success of i.p. injections in these mice. Mice with CPP less than 100 seconds on test day 1 were excluded from all data analysis, including analysis of data collected before CPP was measured (i.e. training performance, locomotor sensitization, and habituation to CPP chambers).

2.4. Procedures for Experiment 2: Effects of cognitive training on daily CPP extinction

2.4.1. Cognitive training—Mice were divided into 4 groups: trained, single rule trained (SRT), yoked to single rule trained (YS), and standard housed (SH) (Fig. 2). Trained mice underwent the same 9-day training protocol described above (**Methods 2.3.2;** Fig. 1; Fig. 2D). SRT mice completed the first 3 days of training in the same manner as trained mice (Fig. 1A–C; Fig. 2C). SRT mice then repeated the compound discrimination session on days 4–9 rather than undergoing set-shifts and reversals (Fig. 2C).

Yoked mice were paired with SRT mice rather than trained mice in order to match the group with the highest number of cereal rewards. Each yoked mouse was paired with one SRT mouse and was placed in a separate training arena during the SRT mouse's sessions (Fig. 2B). Each time the SRT mouse received a cereal reward, a comparable cereal reward was dropped into the arena of the yoked mouse (Fig. 2B). SH mice were food restricted to 85–90% of their *ad libitum* body weight during the cognitive training period but did not receive any training or exposure to the training arena (Fig. 2A).

2.4.2. CPP—CPP habituation, conditioning and testing procedures for Experiment 2 were exactly the same as those for Experiment 1 (**Methods 2.3.4**), with the exception that mice were tested for CPP daily on post-conditioning days 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11. In contrast to the weekly schedule used in Experiment 1, this schedule was designed to promote extinction of CPP (Mueller and Stewart, 2000).

On post-conditioning day 12, mice in Experiment 2 underwent a reinstatement session, in which they received a 5mg/kg priming injection of cocaine (intraperitoneal) followed by a 20-minute test for chamber preference.

2.5. Statistics

For analysis of CPP and locomotion, 2-way repeated measures ANOVAs were performed using time and training as factors. Post-hoc comparisons were performed using Holm-Sidak corrections for multiple comparisons. For analysis of reinstatement data in Experiment 2, a 1-way ANOVA was performed with Holm-Sidak post-hoc comparisons among the groups. For correlations between training performance and CPP levels, the slope coefficient of a linear regression curve fitted to CPP from days 1–14 (the time period in which we observed a difference between trained and yoked animals) was used as a measure of CPP for each mouse. Pearson correlations were performed between training performance and CPP slope

coefficients; a non-parametric Spearman correlation was used for one dataset determined to have a non-normal distribution by a D'Agostino-Pearson omnibus test. Analysis and graphing were performed using GraphPad Prism v6.05.

3. Results

3.1. Experiment 1: Effects of cognitive training on weekly maintenance of cocaine CPP

3.1.1. Cognitive training performance—To test the effects of cognitive training on maintenance of cocaine CPP, we first trained mice on a 9-day cognitive training paradigm in which the mice employed odor-based, texture-based, and spatial rules while digging in scented bedding for food rewards (Fig. 1 and Tables 1–2). The training included three reversals, one intradimensional shift, and two extradimensional shifts. As a control, yoked littermate mice explored identical training arenas while receiving cereal rewards at the same times as trained animals. Standard housed (SH) mice were food restricted in the same manner as trained and yoked mice but did not receive any training or exposure to the training arena. Cognitive training performance, as measured by the fraction of trials resulting in a correct choice in each phase of training, is shown in Fig. S1.

3.1.2. Cognitive training does not affect locomotor sensitization to cocaine—Beginning 4 weeks after completion of cognitive training, we conditioned and tested the mice in a cocaine conditioned place preference (CPP) paradigm (Fig. 3A). During cocaine conditioning, we analyzed the animals' locomotor activity in response to cocaine injections. As expected, mice showed locomotor sensitization across successive cocaine injections (Fig. 3C, $F_{3,81}$=21.34, p<0.0001 for main effect of time; Holm-Sidak adjusted p=0.0002 for day 1 to day 4 in SH animals; Holm-Sidak adjusted p<0.0001 for day 1 to day 4 in yoked animals; Holm-Sidak adjusted p<0.0001 for day 1 to day 4 in trained animals). However, training had no effect on locomotor response to cocaine injections (Fig. 3C; $F_{2,27}$=0.98, p=0.39 for main effect of training; $F_{6,81}$=0.52, p=0.79 for training by time interaction). Trained and yoked mice also did not differ in locomotor activity after injections of saline (Fig. 3B; $F_{2,26}$=0.0021, p=1.00 for main effect of training; $F_{6,78}$=1.16, p=0.34 for training by time interaction).

3.1.3. Cognitive training does not affect initial CPP—After conditioning, we tested mice for their preference for the cocaine- and saline-paired contexts. All groups formed a significant preference for the cocaine-paired context (Fig. 3D, $F_{1,27}$=142.57, p<0.0001 for main effect of cocaine conditioning, Holm-Sidak adjusted p<0.0001 for habituation versus test day 1 within each group). Trained, yoked, and SH animals did not differ in their establishment of CPP on test day 1 (Fig. 3D; $F_{2,27}$=1.07, p=0.36 for main effect of training; $F_{2,27}$=1.10, p=0.35 for training by time interaction).

3.1.4. Cognitive training reduces maintenance of cocaine CPP during weekly testing—Next, to determine the long-term effects of cognitive training on maintenance of cocaine CPP, mice were exposed to the chambers weekly over a 28-day period. Over the course of this 28-day period, trained animals showed reduced CPP compared to yoked and SH controls (Fig. 3E; $F_{2,27}$=5.19, p=0.01 for main effect of training; $F_{8,108}$=2.16, p=0.04 for training by time interaction; $F_{4,108}$=3.24, p=0.02 for main effect of time). In particular, post-

hoc comparisons showed lower CPP in trained animals compared to yoked animals on test days 7 and 14 (Holm-Sidak adjusted p=0.02 for day 7, Holm-Sidak adjusted p=0.04 for day 14), as well as lower CPP in trained animals compared to SH animals on test day 7 (Holm-Sidak adjusted p=0.005). Yoked animals showed significantly lower CPP than SH animals on test day 28 (Holm-Sidak adjusted p=0.02), although yoked animals did not differ significantly from trained animals on test day 28 (Holm-Sidak adjusted p=0.26).

Within-group comparisons across time revealed significant reductions in CPP from test day 1 to test days 7 and 21 in trained animals (Holm-Sidak adjusted p=0.01 for day 1 compared to day 7; Holm-Sidak adjusted p=0.01 for day 1 compared to day 21). In contrast, yoked and SH animals did not show significant changes in CPP from day 1 to any later day (Holm-Sidak adjusted p=0.84, 0.54, 0.84, and 0.14 for days 7, 14, 21, and 28, respectively in yoked animals; Holm-Sidak adjusted p=0.97, 1.00, 0.50, and 1.00 for test days 7, 14, 21, and 28, respectively in SH animals). These data demonstrate that the 9-day cognitive training paradigm employed in these experiments reduced cocaine CPP during a weekly testing period that took place 6–10 weeks after cessation of cognitive training.

3.1.5. Cognitive training performance does not correlate with CPP during weekly testing—

Given that the cognitive training paradigm was designed to engage executive functions such as cognitive flexibility, which might in turn influence drug-seeking behavior, we hypothesized that mice showing the most flexible behavior during cognitive training might show the lowest levels of CPP during the maintenance period. To test this hypothesis, we ran correlation analyses to test whether performance during cognitive training predicted CPP maintenance, as measured by the slope of a linear regression curve fitted to CPP data for each mouse. Using a measure of overall performance, we found no correlation between the fraction of trials that resulted in correct choices across sessions and cocaine CPP (Fig. S2A, r=0.48, p=0.12). Considering each type of rule change separately, we found no correlation between CPP and performance during reversals (Fig. S2B, r=0.06, p=0.85), intradimensional shift (Fig. S2C, r=0.49, p=0.11), or extradimensional shifts (Fig. S2D, r=0.33, p=0.30). Lastly, we considered 2 specific measures of executive function: the animals' ability to inhibit responding to a previously rewarded cue during reversals (perseverative errors), and the animals' ability to maintain responding to a new rule after the first rewarded trial (regressive errors). We found no correlation between either of these measures and CPP (Fig. S2E–F; r=−0.28 and p=0.39 for perseverative errors; r=−0.40 and p=0.19 for regressive errors).

3.2. Experiment 2: Effects of cognitive training on daily extinction of cocaine CPP

To test whether cognitive training could reduce cocaine CPP during a daily extinction testing period, we performed an additional experiment in which mice were tested for CPP on post-conditioning days 1–11. Given the lack of correlation between performance during rule changes and CPP in Experiment 1 (Fig. S2), we further investigated the role of rule changes by including a group of animals that received single rule training (SRT) with no set-shifts or reversals (**Methods 2.4.1;** Fig. 2C). In order to match the large number of cereal rewards received by the SRT mice, the yoked mice were paired with SRT mice rather than trained

mice. The yoked group therefore differed in Experiments 1 and 2; yoked mice in Experiment 2 are designated YS (i.e. yoked to SRT) in Fig. 2 and Fig. 4.

3.2.1. Cognitive training does not affect locomotor sensitization to cocaine or initial establishment of CPP—In accordance with the results of Experiment 1, we observed locomotor sensitization to cocaine (Fig. 4C; $F_{3,87}$=21.95, p<0.0001 for main effect of time; Holm-Sidak adjusted p=0.003, p=0.0002, p<0.0001, and p=0.02 for injection 1 compared to injection 4 in SH, YS, SRT, and trained mice, respectively) but no effect of training on locomotor responses to cocaine ($F_{3,29}$=1.20, p=0.33 for main effect of training; $F_{9,87}$=0.46, p=0.90 for training by time interaction). Training also had no effect on locomotor responses to saline injections (Fig. 4B; $F_{3,29}$=0.79, p=0.51 for main effect of training; $F_{9,87}$=1.12, p=0.36 for training by time interaction). Similarly, we observed establishment of CPP in all groups (Fig. 4D; $F_{1,29}$=270.72, p<0.0001 for main effect of time; Holm-Sidak adjusted p<0.0001 for habituation to test day 1 within each group), with no effect of training on initial establishment of CPP ($F_{3,29}$=0.71, p=0.55 for main effect of training; $F_{3,29=}$0.97, $p_{=}$0.42 for training by time interaction).

3.2.2. Trained and yoked mice show extinction of CPP, while standard housed mice show no extinction of CPP—In contrast to our weekly testing paradigm, we observed no main effect of training on CPP during the daily extinction testing period (Fig. 4E; $F_{3,29}$=0.46, p=0.71). We did, however, observe a training by time interaction ($F_{30,290}$=1.72, p=0.01). To investigate the nature of this interaction, we performed within-group comparisons of CPP from day 1 to each later day. SH mice showed no reduction in CPP from test day 1 to any later test day, while YS, SRT, and trained mice all showed reductions in CPP from test day 1 to later test days (Fig. 4H).

In order to test whether cognitive training altered reinstatement of CPP, we exposed the mice to the CPP chamber after a cocaine priming injection (5mg/kg i.p.) on post-conditioning day 12. Cognitive training had no effect on reinstatement, as measured by CPP on reinstatement day normalized to habituation day (Fig 4F; $F_{3,28}$=1.25, p=0.31) or as measured by CPP on reinstatement day minus CPP on test day 11 (Fig 4G; $F_{3,28}$=0.09, p=0.97).

4. Discussion

4.1. Experiment 1: Effects of cognitive training on weekly CPP maintenance

Our results from Experiment 1 show that 9 days of cognitive training reduced maintenance of cocaine CPP compared to standard housed and yoked control conditions in a weekly testing paradigm (Fig. 3E). Locomotor sensitization during conditioning and initial CPP on test day 1 were comparable between the trained and control groups (Fig. 3B–D), but the groups differed in their behavior when the drug was no longer delivered. Importantly, the CPP testing period in which we saw a beneficial effect of training took place 6–10 weeks after cessation of the training, suggesting that brief cognitive training may carry long-term benefits for reducing drug-seeking behavior.

Exposure to drug-associated contexts can elicit both reconsolidation and extinction of drug-context associations. Previous literature shows that infrequent (i.e. biweekly) CPP testing supports long-term maintenance of CPP, potentially by allowing for reconsolidation to strengthen the drug-context association, while daily testing promotes extinction of CPP (Mueller and Stewart, 2000). In alignment with these previous findings, YT and SH animals in our weekly testing paradigm did not show significantly reduced CPP from test day 1 to any later test day. Cognitive training may have altered reconsolidation processes, leading to a weakening of the drug-context association during the testing period. Alternatively, cognitive training may have promoted extinction learning, allowing mice to more quickly form a new association between the CPP context and the absence of drug.

In addition to reconsolidation and extinction, previous literature shows increases in drug-seeking behavior over time, termed incubation, in abstinent rats with a history of cocaine self-administration (Grimm et al., 2001). Although we did not observe statistically significant increases in CPP from day 1 to any later day, it is possible that cognitive training interfered with an incubation-like effect occurring along with reconsolidation and/or extinction during the weekly CPP testing period. Whether cognitive training primarily alters reconsolidation, extinction, or incubation, the results from Experiment 1 suggest that cognitive training may hold long-term benefits for reducing maintenance of drug-context associations.

Although the cognitive training paradigm involved brief periods of environmental enrichment, as animals explored an arena and received cereal rewards during training, YT control mice received this same enrichment. The beneficial effect of cognitive training on CPP maintenance is therefore not explained by a simple enrichment effect. Furthermore, our results diverge from environmental enrichment literature in that we observed a persistent effect of cognitive training during a testing period in which animals remained in relatively deprived housing conditions (i.e. standard laboratory housing) with no further cognitive training. This is in contrast to studies of environmental enrichment in which animals returning to standard housing after a history of enrichment show no reduction in drug-seeking (Chauvet et al., 2012) or exacerbation of drug-seeking (Nader et al., 2012) compared to animals that never experienced enrichment. For animals that experience relatively few opportunities for challenge or learning in their housing environments, engaging in cognitive training may induce long-term changes in neural circuitry underlying appetitive behaviors. These long-term changes may in turn influence later responses to future challenges such as exposure to drugs and drug-associated contexts.

Our results parallel environmental enrichment literature in that both training and enrichment reduce drug-seeking behavior specifically when the drug is no longer delivered, suggesting that both enrichment and cognitive training may alter animals' responses to drug-associated cues in the absence of the drug itself (Chauvet et al., 2012; Chauvet et al., 2009; Thiel et al., 2009). Interestingly, environmental enrichment also reduces sucrose-seeking behavior specifically when sucrose is no longer delivered, suggesting a potentially broad effect of enrichment and/or cognitive training on animals' ability to respond adaptively when contingencies change (Grimm et al., 2008).

When we looked more deeply into performance in the trained group, quantifying individual differences in performance during the different phases of cognitive training, we did not find any relationship with individual differences in CPP scores during weekly maintenance tests (Fig. S2). This result suggests that engaging with cognitively challenging tasks, even with varying levels of success or prior ability, may hold benefits for reducing future maintenance of drug-context associations.

The learning paradigm we employed was complex, involving multiple sensory modalities and two distinct types of rule changes. Even before experiencing rule changes, mice had an opportunity to choose among multiple options, receive feedback, and learn an abstract rule based on cues from two sensory modalities. This active and complex learning experience contrasts starkly with standard laboratory housing conditions, in which mice experience few opportunities to learn new contingencies. One explanation for the beneficial effect of training is that set-shifts and reversals caused mice to develop executive functions such as cognitive flexibility and inhibitory control of behavior, which predict resilience to drug-seeking behavior in both humans and animal models (Aytaclar et al., 1999; Belin et al., 2008; Economidou et al., 2009; Perry et al., 2005; Tarter et al., 2003). Given the lack of correlation between performance during rule changes and CPP (Fig. S2), however, it is also possible that rule changes were unnecessary for the beneficial effect of training. The training paradigm included many opportunities to make choices and learn about different features of the task, and a simpler version of the task may have been sufficient to reduce maintenance of cocaine CPP. Future studies could investigate whether rule changes were necessary for the beneficial effects of cognitive training on CPP maintenance in Experiment 1.

4.2. Experiment 2: Effects of cognitive training on daily CPP extinction

In Experiment 2, we tested the effects of cognitive training and yoked rewards on CPP extinction using a daily testing schedule. All groups that had previous experience in which they had the opportunity to leave their home cages to receive rewards in a training arena showed significant extinction of CPP, while standard housed cage mates that remained in their home cages during the training period did not show extinction of CPP (Fig. 4H). This result suggests that brief interventions, involving either cognitive training or receipt of rewards in a novel context, may hold long-term benefits for the extinction of drug-context associations.

As in Experiment 1, the effect of interventions observed in Experiment 2 may have been mediated by extinction learning or reconsolidation. Mice that left their home cages for rewards in a training arena may have been better able to learn a new association between the CPP context and the absence of drug during the extinction period. Alternatively, the cognitive training and yoked interventions may have altered reconsolidation, such that mice with prior experiences of reward in a training arena engaged in less strengthening or more weakening of the drug-context association upon re-exposure to the CPP context.

In contrast to Experiment 1, the yoked control condition (YS, i.e. yoked to SRT) in Experiment 2 appeared to be just as beneficial as the trained and SRT conditions. Importantly, YS animals in Experiment 2 were paired with SRT rather than trained animals in order to match the higher number of rewards received by SRT animals. Due to the

repetitive nature of the SRT task, which involved the same compound discrimination session on days 3–9, YS animals in Experiment 2 experienced a more predictable schedule of rewards than YT (i.e. yoked to trained) animals in Experiment 1. YS animals in Experiment 2 may therefore have learned associations between sensory stimuli associated with the SRT mouse's performance (e.g. the opening and closing of the start gate or the sounds of digging) and the delivery of cereal rewards. This predictable schedule of reward delivery, combined with potential Pavlovian learning related to task-relevant sensory stimuli, may have influenced YS mice's later formation of cue-reward associations in the CPP paradigm. Thus, although their receipt of rewards was not contingent on learning an odor, texture, or spatial association, YS animals may still have experienced unintended contingency learning that influenced their later behavior in the CPP paradigm. Future experiments could elucidate the potential effects of predictable and unpredictable schedules of reward delivery on CPP extinction.

Although we observed a training by time interaction and within-group reductions in CPP during the extinction period (Fig. 4E, H), comparisons among the groups at each time point did not yield statistically significant differences. With 4 groups of mice tested at 11 time points, our statistical power was limited for detecting differences among the groups at individual time points. Future studies may help to clarify whether, for example, training with rule changes held benefits beyond those of single rule training.

After the CPP extinction period, we tested the 4 groups of mice for reinstatement of CPP in response to a cocaine priming injection. No benefits of training were observed in the reinstatement assay (Fig. 4F, G). However, the lack of CPP extinction in SH animals may have interfered with this measure. Further experiments will be needed to clarify whether training or predictable reward delivery affect reinstatement of cocaine CPP.

In addition to the relative deprivation of standard laboratory housing, SH animals experienced other potential stressors along with their trained cage mates during the training period. All groups of mice were food restricted, transported to and from the training room, and exposed to light during their dark cycle. Cognitive training or predictable reward delivery therefore may have counteracted not only the effects of environmental deprivation (i.e. standard laboratory housing), but also the effects of these additional stressors.

4.3. General Discussion

Conditions of deprivation, including standard laboratory housing, are known to promote drug-seeking behavior in animal models of substance use disorders. Here, we tested the long-term effects of brief interventions on drug-seeking behavior after animals returned to deprived standard housing conditions. We found that cognitive training induced long-term reductions in CPP maintenance, above and beyond the effects of receiving yoked rewards in a training arena. In tests of CPP extinction, we found that a brief period of either learning rules or receiving predictable yoked rewards in a training arena supported CPP extinction. Taken together, these results suggest that brief interventions may buffer the effects of adversity and deprivation, promoting long-term resilience to drug-seeking behavior.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Cognitive training reduces maintenance of cocaine CPP during weekly testing.
- Cognitive training or predictable reward delivery promotes daily CPP extinction.
- Beneficial effects of these brief interventions on CPP last at least 6 weeks.

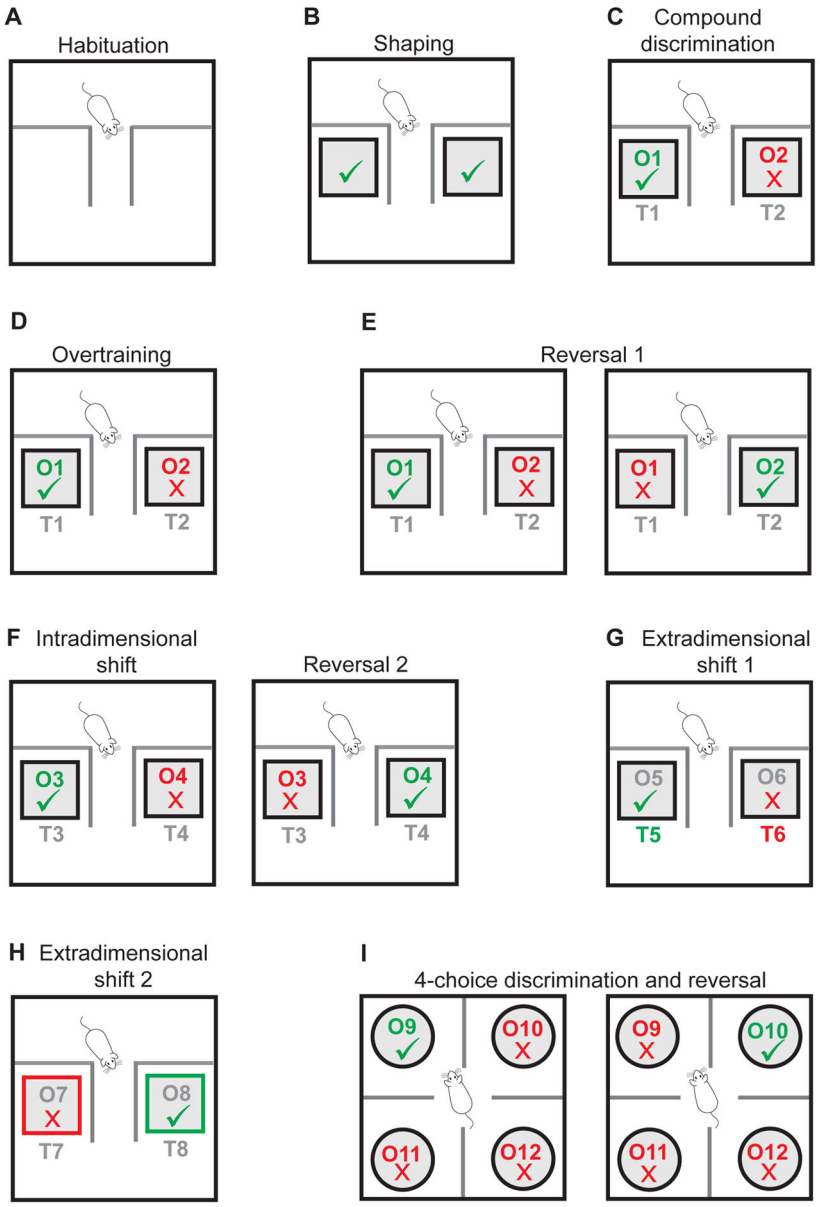

Fig. 1. Schematic of cognitive training paradigm

Each subfigure (**A–I**) shows 1 day of training, with 9 total days. The mouse is shown in the start compartment of the training arena. Digging pots are shown by filled rectangles (**B–H**) or filled circles (**I**). The digging pot containing an accessible cereal reward is indicated by a check mark and green color on the relevant exemplar, while incorrect choices are indicated by an "x" and red color on the relevant exemplar. Irrelevant exemplars are shown in gray. O1-12 refer to the 12 odors used to scent the digging medium; T1-8 refer to the 8 textures used to cover the digging pots. To control for odor, all texture pairs were of the same material with discriminable textures on opposing sides (e.g. sand paper, reverse sandpaper). The location of each odor/texture combination was pseudorandomized between trials. (**A**) Day 1 (habituation): mice were exposed to cereal pieces in the arena. (**B**) Day 2 (shaping): mice learned to dig in wood shavings for cereal rewards. (**C**) Day 3 (compound

discrimination): 1 of 2 odors was rewarded, while 2 textures were irrelevant (20 trials). (**D**) Day 4 (overtraining): the same rule used on Day 3 was repeated for 30 additional trials. (**E**) Day 5 (reversal 1): the same odors/textures were used as on days 3 and 4. The rule used on days 3 and 4 was repeated for the first 10 trials, after which the opposite odor was rewarded for 40 trials. (**F**) Day 6 (intradimensional shift and reversal 2): for the first 20 trials, an odor-based rule was employed with 2 novel odors and 2 novel textures. For last 35 trials, the opposite odor was rewarded. (**G**) Day 7 (extradimensional shift, texture): a texture-based rule was employed with 2 novel odors and 2 novel textures (30 trials). (**H**) Day 8 (extradimensional shift, spatial): a spatial rule was employed with 2 novel odors and 2 novel textures. One location (i.e. the digging pot on the left side) was rewarded, regardless of odor and texture (25 trials). (**I**) Day 9 (4-choice discrimination and reversal 3): 4 novel odors were used. One odor was rewarded for the first 20 trials, and a different odor was rewarded for the last 25 trials.

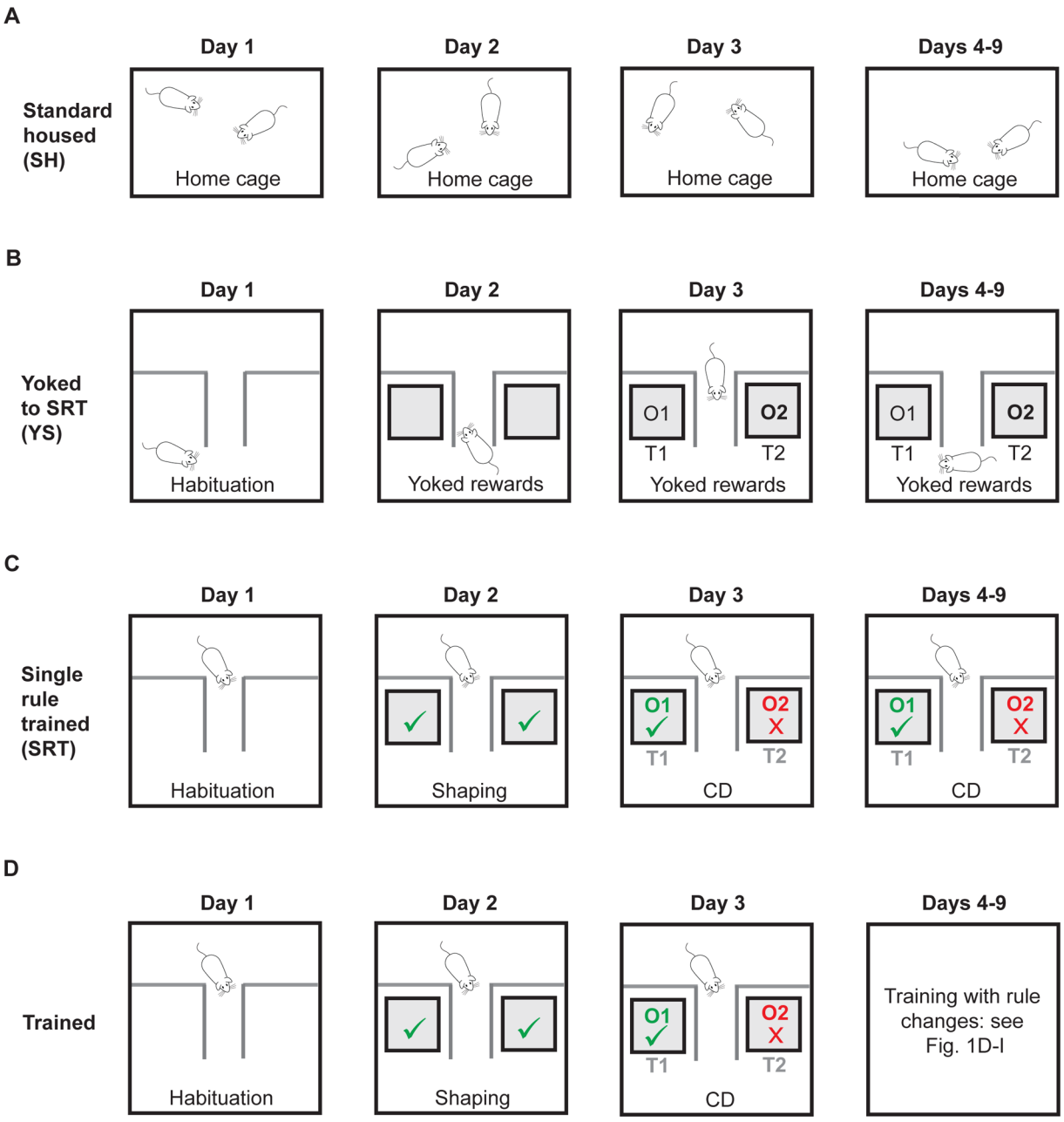

Fig. 2. Schematic of experimental groups for Experiment 2

Mice are shown in the home cage (**A**) or in a training arena (**B–D**). Digging pots containing scented bedding are shown by filled rectangles. The digging pot containing an accessible cereal reward is indicated by a check mark and green color on the relevant exemplar, while incorrect choices are indicated by an "x" and red color on the relevant exemplar. Irrelevant exemplars are shown in gray. O1 and O2 refer to the 2 odors used to scent the digging medium; T1 and T2 refer to the 2 textures used to cover the digging pots. To control for odor, texture pairs were of the same material with discriminable textures on opposing sides (e.g. velvet, reverse velvet). (**A**) Standard Housed (SH) mice remained in their home cages

with no training or cereal rewards. (**B**) Each yoked mouse was paired with one mouse that underwent single rule training (SRT). The yoked mouse explored an identical arena adjacent to the SRT mouse and received a cereal reward each time the SRT mouse earned a cereal reward. (**C**) On days 1–3 of training, single rule trained (SRT) mice underwent habituation, shaping, and compound discrimination (CD) as described in Fig. 1A–C. On days 4–9 of training, SRT mice repeated the compound discrimination task with no rule changes. The location of each odor/texture combination was pseudorandomized between trials. (**D**) Trained mice underwent the same procedures shown in Fig. 1A–I, including set-shifts and reversals. The location of each odor/texture combination was pseudorandomized between trials.

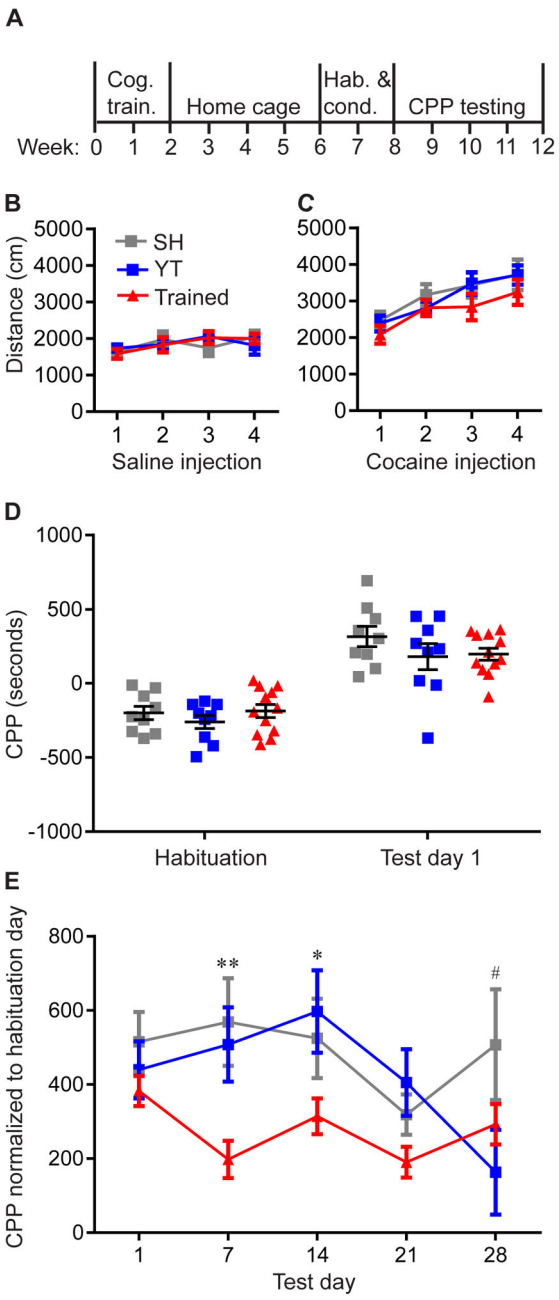

Fig. 3. Cognitive training reduces maintenance of cocaine CPP without altering locomotor sensitization or initial CPP levels

(**A**) Experiment timeline. “Cog. train.” is cognitive training, “Hab. & cond.” is habituation and conditioning (i.e. habituation to CPP chambers followed by cocaine conditioning, see **Methods 2.3.4**). Mice were ages p55–p77 at the start of cognitive training (day 0 on the timeline). (**B**) Cognitive training did not alter locomotor activity recorded during 15-minute sessions after saline injections. *N*=11 trained mice (1 mouse excluded due to equipment failure), *N*=9 yoked to trained (YT) mice, *N*=9 standard housed (SH) mice. (**C**) Cognitive training did not alter locomotor activity recorded during 15-minute sessions after cocaine

injections. All groups showed increased locomotor activity across successive cocaine injections, consistent with sensitization. *N*=12 trained mice, *N*=9 YT mice, *N*=9 SH mice. (**D**) Cognitive training did not alter the initial establishment of cocaine CPP, as measured by seconds in the cocaine-paired chamber minus seconds in the saline-paired chamber. CPP is shown before and after cocaine conditioning (i.e. on habituation day and on test day 1). *N*=12 trained mice, *N*=9 YT mice, *N*=9 SH mice. (**E**) Cognitive training reduced maintenance of CPP over a 28-day testing period. CPP values on test days were normalized to each mouse's CPP value on habituation day. *N*=12 trained, *N*=9 YT mice, *N*=9 SH mice. **p*<0.05; ***p*<0.01 for trained compared to YT or SH animals. #p<0.05 for YT compared to SH animals. All bars represent means; all error bars represent SEM.

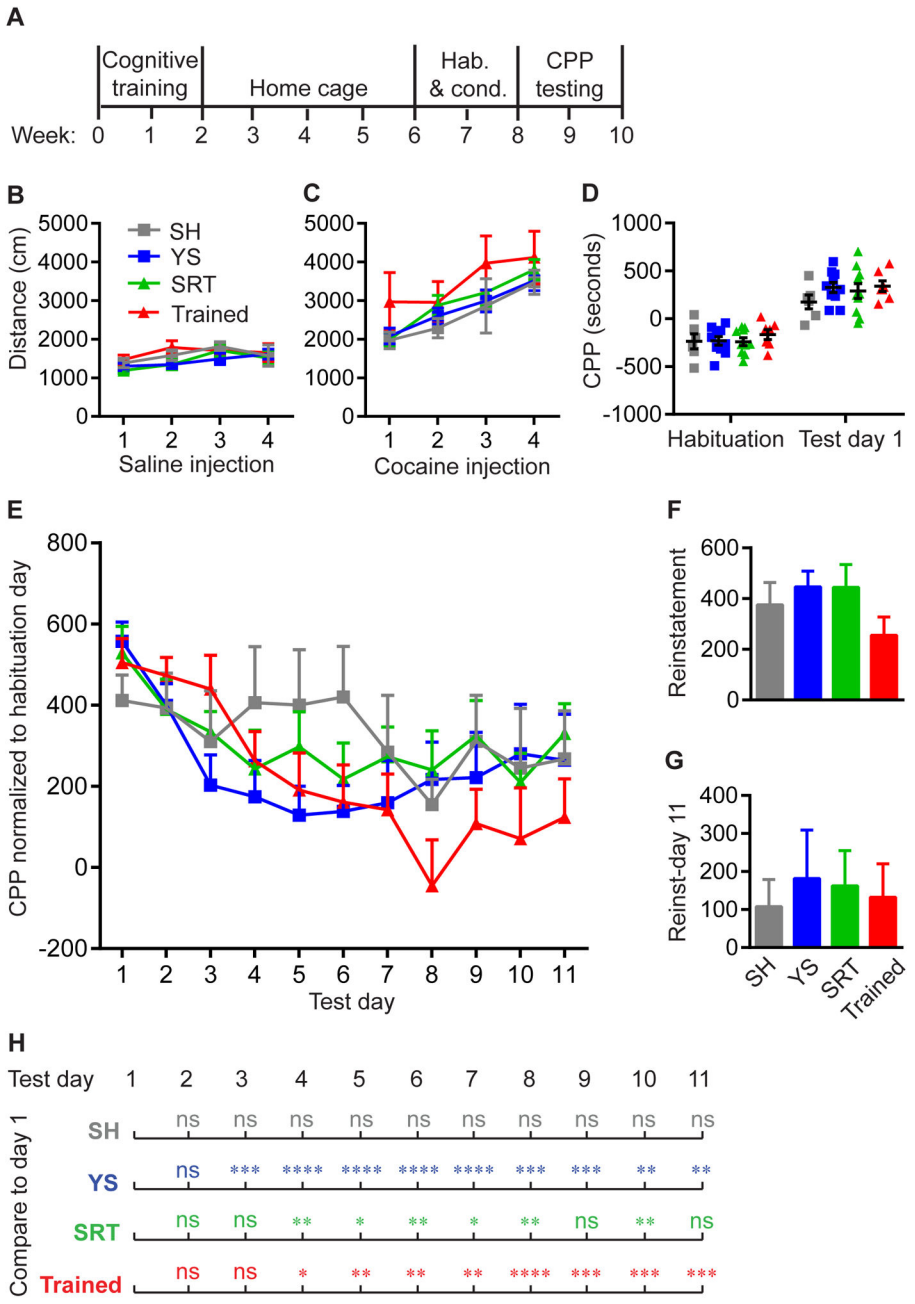

Fig. 4. Trained and yoked mice show extinction of CPP, while standard housed mice show no extinction of CPP

(**A**) Experimental timeline. “Hab. & cond.” is habituation and conditioning (i.e. habituation to CPP chambers followed by cocaine conditioning). (**B**) Cognitive training did not alter locomotor activity recorded during 15-minute sessions after saline injections. “SH” is standard housed. “SRT” is single rule trained. “YS” is yoked to single rule trained. *N*=6 SH mice, *N*=10 YS mice, *N*=10 SRT mice, *N*=7 trained mice. (**C**) Cognitive training did not alter locomotor activity recorded during 15-minute sessions after cocaine injections. All groups showed increased locomotor activity with successive cocaine injections, consistent with sensitization. *N*=6 SH mice, *N*=10 YS mice, *N*=10 SRT mice, *N*=7 trained mice. (**D**)

Cognitive training did not alter the initial establishment of cocaine CPP, as measured by seconds in the cocaine-paired chamber minus seconds in the saline-paired chamber. CPP is shown before and after cocaine conditioning (i.e. on habituation day and on test day 1). *N*=6 SH mice, *N*=10 YS mice, *N*=10 SRT mice, *N*=7 trained mice. (**E**) Trained, SRT, and YS animals showed significant within-group reductions in CPP across the 11-day CPP extinction period, while SH animals showed no reduction in CPP. Statistics for within-group comparisons across time are shown in **H**. *N*=6 SH mice, *N*=10 YS mice, *N*=10 SRT mice, *N*=7 trained mice (**F**) Cognitive training did not affect reinstatement, as measured by CPP on reinstatement day minus CPP on habituation day. *N*=6 SH mice, *N*=10 YS mice, *N*=9 SRT mice (1 outlier excluded), *N*=7 trained mice. (**G**) Cognitive training did not affect reinstatement, as measured by CPP on reinstatement day minus CPP on test day 11. *N*=6 SH mice, *N*=10 YS mice, *N*=9 SRT mice (1 outlier excluded), *N*=7 trained mice. (**H**) Trained, SRT, and YS animals showed extinction of CPP, as evidenced by significant reductions in CPP from day 1 to later test days. SH animals did not show extinction of CPP. Asterisks indicate significance levels for Holm-Sidak adjusted p values. *N*=6 SH mice, *N*=10 YS mice, *N*=10 SRT mice, *N*=7 trained mice. *$p<0.05$; **$p<0.01$; ***$p<0.001$; ****$p<0.0001$. All bars represent means; all error bars represent SEM.

Table 1

Exemplar combinations used during the 2-choice phase of cognitive training

Two acrylic digging pots were wrapped in textured covering and filled with scented wood shavings. One exemplar was rewarded in each session.

Training phase	Odor A	Odor B	Texture A	Texture B	Rewarded exemplar
Compound discrimination	Vanilla	Cinnamon	Velvet	Reverse velvet	Vanilla
Overtraining	Vanilla	Cinnamon	Velvet	Reverse velvet	Vanilla
Reversal 1	Vanilla	Cinnamon	Velvet	Reverse velvet	Cinnamon
Intradimensional shift	Almond	Coconut	Diaper	Reverse diaper	Almond
Reversal 2	Almond	Coconut	Diaper	Reverse diaper	Coconut
Extradimensional shift (texture)	Lemongrass	Orange	Sandpaper	Reverse sandpaper	Sandpaper
Extradimensional shift (spatial)	Lavandin	Wintergreen	Pleather	Reverse pleather	Left side

Table 2

Exemplar combinations used during the 4-choice phase of cognitive training

Four ceramic digging pots were filled with scented wood shavings, and one odor was rewarded in each session.

Training phase	Odor A	Odor B	Odor C	Odor D	Rewarded odor
4-choice discrimination	Anise	Clove	Thyme	Litsea	Anise
Reversal 3	Anise	Clove	Eucalyptus	Thyme	Clove